contain the phrase "high percentage of the tumor cells". It is respectfully submitted that, as amended, claim 1 particularly points out and distinctly claims the subject matter which Applicant regards as the invention. It is therefore respectfully requested that the rejection of claim 1 under 35 USC 112, second paragraph, be withdrawn.

Claim 1 has been rejected under 35 USC 112, first paragraph, on the basis that it does not reasonably provide enablement for a solid non-lymphoid primary tumor cell with infiltrating lymphocytes, the Examiner stating that the claim does not exclude solid non-lymphoid primary tumors which contain tumor infiltrating lymphocytes, and that tumor infiltrating lymphocytes are usually indicative of a positive immune response of the host against the tumor and do not correlate with increased metastatic potential.

Claim 1, as amended, recites determining the percentage of "tumor cells" of each of the samples which express said at least one product. Thus, claim 1, as amended, excludes the counting of lymphocytes or other leukocytes since it is only tumor cells that are counted. A discussion of various methods for insuring that tumor cells and not lymphocytes or other leukocytes are counted is contained in paragraph 5.1.0 on page 8 and paragraph 7.1 on page 14 of the specification. As discussed therein, leukocytes include lymphocytes. One method would be to double-label the cells with both T-cell markers and epithelial cell markers, as illustrated in FIG. 22B and discussed in paragraphs 5.1.0 and 6.0, so that only the cells identified by the epithelial cell markers (tumor cells) are counted. Another method would be to treat the cells to remove any leukocytes therefrom using a process as discussed in the above paragraphs of the specification before determining the percentage of the non-leukocyte or tumor cells of each treated sample which express the product. It is

therefore respectfully submitted that one of ordinary skill in the art would know how to use the instant invention to predict the metastatic potential of the primary tumor in the presence of tumor infiltrating lymphocytes and that the specification therefore provides enablement therefor. It is therefore respectfully requested that the rejection under 35 USC 112, second paragraph, be withdrawn.

Claim 1 has also been rejected under 35 USC 103(a) as not being patentable over Kim et al (Proc. Am. Assoc. Cancer Res., 1988, vol. 29, p.67) in view of U.S. patent 5,536,642 to Barbera-Guillem et al, the Examiner stating that Barbera-Guillem et al teaches that T-cell receptor beta is expressed on non-lymphoid tumors, and the decreased expression of T-cell receptor beta on said tumor is indicative of a positive response after anti-cancer therapy. The Examiner further states that it would not have been unobvious to correlate the expression of T-cell beta antigen on non-lymphoid tumors with metastatic potential and that one of ordinary skill in the art would have been motivated to do so with a reasonable expectation of success by the teachings of Kim et al (1988) on the increased metastatic potential of rat mammary carcinoma cells when expressing T-cell antigens.

For the reasons provided hereinafter, it is respectfully submitted that claim 1, as amended, is unobvious over the references of record and therefore patentable.

In accordance with the present invention, a plurality of representative samples of a solid non-lymphoid primary tumor are obtained, and the percentage of tumor cells of each of the samples which express one or more of certain products associated with the T-cell is determined in order to predict the lymphotropic metastatic potential of the tumor. If no tumor cells in all of the samples are detected to express such product or products, the metastatic potential of the as yet

nonmetastasized primary tumor is predicted to be low.

Five specific T-cell associated products (TCRβ, CD3, CD4, CD8, and ZAP-70) have been identified by both clinical and experimental testing to show a high correlation between future metastasis when present in cancer cells and future non-metastasis when not found to be present in cancer cells. These products are thus high predictors of potential or future metastasis when found in as yet nonmetastasized cancer cells. This predictability thus confirms Applicants' belief that the acquisition of lymphotropic metastasis by cancer cells is accompanied by their ability to express aberrant lymphoid specific genes or their products, i.e., a relation between T cells (which are always migratory) and cancer cells which become metastatic (invasive cancer cells which become migratory). See page 1, last full paragraph, of the specification. Thus, by looking for and finding one or more of such products associated with the T cell in a cancer cell, one can predict potential or future metastasis of a primary (as yet nonmetastasized) tumor.

The clinical and experimental data is discussed in detail in the previous Amendment. The clinical findings, as corroborated by the experimental data, clearly show that the detection of the presence of the T-cell associated products CD3, CD4, CD8, TCRβ (CTβ), and ZAP-70 expressed by tumor cells in a solid, non-lymphoid primary tumor may be a predictor of potential or future metastasis and that their absence (non-detection) may be a predictor of potential or future nonmetastasis.

Barbera-Guillem et al discloses the use of measurement of cell-associated interleukin-2 receptor alpha (IL-2R alpha) expression in solid, non-lymphoid tumors in prognosing metastatic potential of the tumor and in monitoring the efficacy of anticancer therapy against metastatic cells of non-lymphoid tumors. Barbera-Guillem et al also discloses that human and

experimental non-lymphoid tumors express TCRβ and that clinical applications of the cell-associated expression of TCRβ by human solid non-lymphoid tumors are (1) immune modulation, and (2) TCRS-targeted antineoplastic drug therapy including the use of the tumor specific TCRβ phenotype in monitoring the efficacy of anticancer treatment against non-lymphoid tumors. Barbera-Guillem et al further discloses that the efficacy of such treatment may be monitored by measuring the cell-associated expression of TCRβ, wherein a decrease in the number of non-lymphoid tumor cells expressing cell-associated TCRβ relative to the number of non-lymphoid tumor cells expressing TCRβ prior to initiation of the therapy is indicative of an anticancer effect from the therapy.

Efficacy of therapy (which involves primary or secondary tumor growth or shrinkage) should not be correlated with prediction of future metastasis (the spread of primary tumor cells to distant sites to form secondary tumors). The determination of the efficacy of a therapy of either a primary or secondary tumor by the reduction of certain markers does not mean that the markers are also useful as a predictor of future metastasis. In this regard, Barbera-Guillem et al discloses the use of a measurement of IL-2R alpha expression in solid non-lymphoid tumors in two different categories, i.e., (1) prognosing the metastatic potential of the tumor, and (2) monitoring efficacy of anticancer therapy. However, contrary to the present invention, Barbera-Guillem et al does not disclose the use of measurement of TCRβ expression in primary solid non-lymphoid tumors in prognosing the metastatic potential of the tumor, as provided by the present invention. See the Abstract thereof.

Kim et al (which is cumulative to the Kim et al reference applied in the previous Office Action dated March 23, 2000, and cited in the Information Disclosure Statement as no. 2) discloses

the results of fusing *in vitro* a nonmetastasizing rat mammary tumor cell line with thymocytes to test the notion that lymphogenously metastasizing carcinoma cells might have acquired the invasive and migratory property through somatic hybridization with lymphocytes during various immunoselection processes encountered in their development period over the lifetime of patients. *In vivo*, about 16 to 25 per cent of the fused cells metastasized. It was stated that the cells expressed thymocyte-specific products. It was concluded that the conversion of nonmetastasizing cells to metastasizing ones was achieved by somatic hybridization of tumor cells with lymphocytes.

The tumors discussed in Kim et al are artificially produced and would thus be expected to have products of the thymocytes from which they were fused. However, the fact that something can be artifically produced does not mean that it would be found in nature. Moreover, a mouse model does not necessarily reflect the situation in humans. The clinical as well as experimental data disclosed in the specification as well as discussed in the previous Amendment confirms that the detection of the presence of one or more of certain specific ones (CD3, CD4, CD8, TCRβ (CTβ), and ZAP-70) of T-cell associated products expressed by tumor cells in a solid, non-lymphoid primary tumor may be a predictor of potential or future metastasis and that their absence (non-detection) may be a predictor of potential or future nonmetastasis. While TCRβ is discussed in Barbera-Guillem et al, contrary to the present invention, it is not discussed therein as being a predictor of potential or future metastasis.

The fact that only about 1 in 4 (or less) of the implanted nodules of the fused cells of Kim et al were consistently metastatic would not suggest that the detection of the products of the thymocytes would be predictive of metastasis. On the contrary, Kim et al teaches away from the present invention by

its teaching that such a small percentage of the nodules were consistently metastatic.

Neither Barbera-Guillem et al or Kim et al or any other of the references of record, whether taken together or individually, discloses, teaches, or suggests a method of predicting the lymphotropic metastatic potential of a solid non-lymphoid primary tumor wherein the percentage of tumor cells of each of representative samples of the tumor which express one or more of the products TCRβ, CD3, CD4, CD8, and ZAP-70 is determined, wherein the metastatic potential of the tumor is predicted to be low when no tumor cells in all of the samples are detected to express any of the products, as claimed in claim 1, as amended, so that a prediction may be reliably made as to whether a primary (localized) tumor will (in the future) metastasize to distant sites. Therefore, it is respectfully submitted that claim 1, as amended, is unobvious over the prior art and therefore patentable.

It is therefore respectfully submitted that this application is in condition for allowance, and such is respectfully requested. If it would aid in advancing this application to issue, the Examiner is respectfully urged to call the undersigned attorney for Applicant at the number below.

Respectfully submitted,

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Enclosure

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